FIG. 1 Context-Sensitive Parallel Optimization

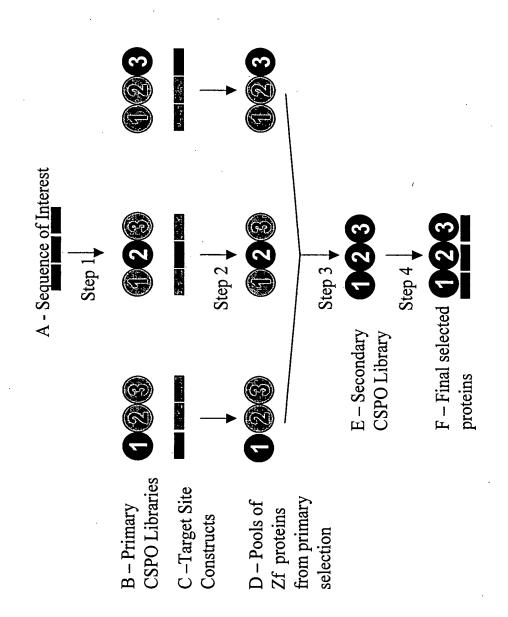


FIG. 2
Construction of Randomly Recombined Libraries

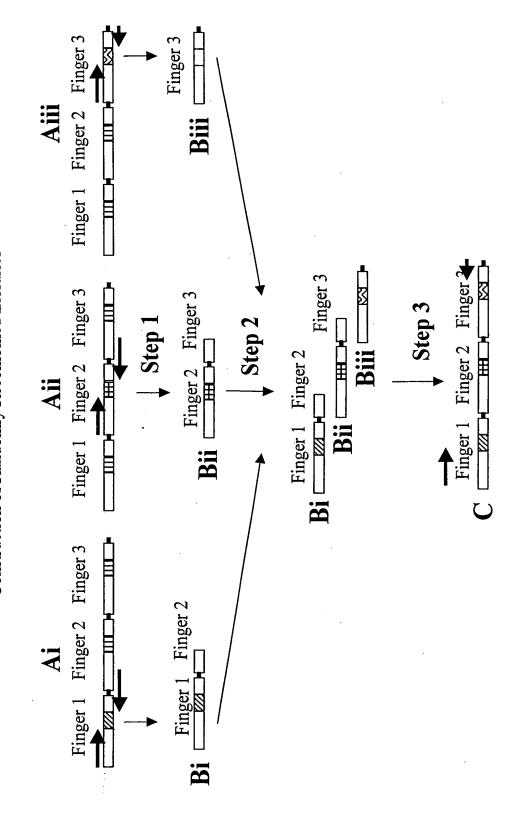


FIG. 3 Quantifying Affinity of ZFPs



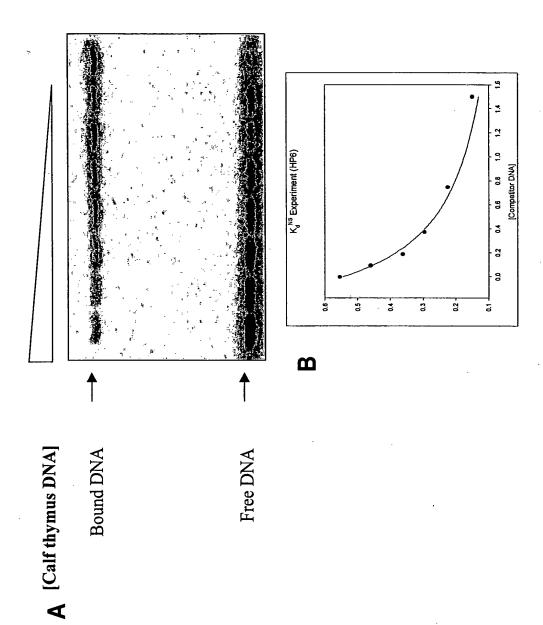


FIG. 5

Validating Context-Sensitive Parallel Optimization

i) BCR-ABL s'GCAG

s'GCAGAAGCC³

ii) erb-B2

s'GCCGCAGTG3'

iii) HIV promoter

5'GATGCTGCA3'

DRSSTR QGGNVR QAATQR*

erb-B2

::

RKDSVR QSGDRR DCRDAR*

iii) HIV promoter

ASADTR NRSDSR TSSNKK#

B

BCR-ABL

FIG. 6
Selections for the BCR-ABL site

CCG AAG ACG ← DNA target site F1 F2 F3

-1 12356-1 12356-1 12356

		-000711	1 15300-1 15300-1 1-05641	000711	_
	"wild-type"	DRSSTR	DRSSTR QGGNVR QAATQR	QAATQR	
4	BCAB1	DSPTRR	DSPTRR QGANRR QANTQR	QANTQR	
	BCAB2	DSPTRR	dsptrr ontntr oantor	QANTQR	
	BCAB3	DSPTRR	DSPTRR QNTNTR QANTQR	QANTQR	
	BCAB4	DESTRR	Destrr Qgpnrr orntor	QRNTQR	
٠	BCAB5	DSPTRR	DSPTRR QGPNRR QGNTTR	QGNTTR	
	BCAB6	DSPTRR	DSPTRR QNPNVR QRNTQR	QRNTQR	
4	BCAB7	DSPTRR	DSPTRR QRTNIR QRNTQR	QRNTQR	
\	BCAB8	DRPTRR	DRPTRR QGANRR QANTQR	QANTQR	
	BCAB9	DSPTRR	DSPTRR QNTNNR QANTQR	QANTQR	
	BCAB10	DSPTRR	DSPTRR QKPNDR QGNSIR	QGNSIR	
	BCAB11	DSPTRR	DSPTRR QSTNNR QGNSNR	QGNSNR	
	BCAB12	DSPTRR	dsptrr onvntr ornior	QRNTQR	

fold-activation

FIG. 7 In vitro characterization of BCR-ABL ZFPs

Protein	ន្ត	Sequence	K _{spec} (pM)	K _{non-spec} Sp (nM)	Specificity ratio	# of DNA bases specified
"wt"	DRSSTR	QGGNVR QAA	DRSSTR QGGNVR QAATQR 28 (±3.9)	55 (±12)	1,980	R
BCAB 1	DSPTRR	QGANRR QAN	DSPTRR QGANRR QANTQR 78 (±13)	2100(±270) 27,000	27,000	~7.4
BCAB 7	DSPTRR	QRTNIR QRN	DSPTRR QRTNIR QRNTQR 60 (±8.5)	1300 (±97)	23,000	~7.2
Zif268	·		8.1(±1.8)	8.1(±1.8) 1000(±120) 130,000	130,000	8

FIG. 8

Selections for the erb-B2 site

GTG ACG CCG DNA target site F1 F2 F3

-1 12356 -1 12356 -1 12356

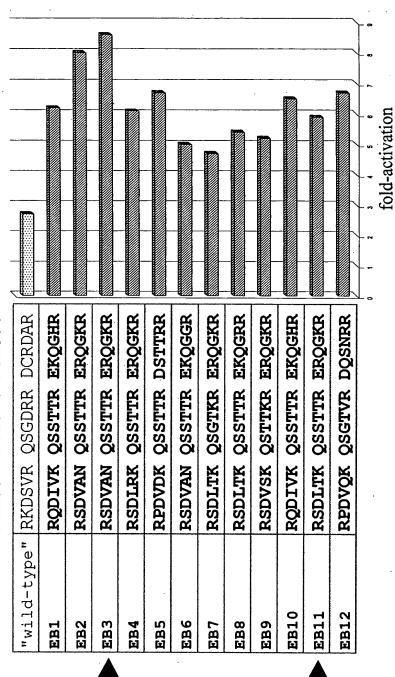


FIG. 9
In vitro characterization of erb-B2 ZFPs

Protein	Sequence	K _d spec (pM)	K _d non-spec S <u>r</u> (nM)	ecificity ratio	# or Specificity DNA bases ratio specified
"wt"	RKDSVR QSGDRR DCRL	QSGDRR DCRDAR 150 (±23)	1000 (±120)	6,700	~ 6.4
西 田 3	RSDVAN QSSTTR ERQGKR 31(±3.1)	KR 31 (±3.1)	1100 (±15)	35,000	~7.5
EB 11	RSDLTK QSSTTR ERQG	QSSTTR ERQGKR 65 (±3.9)	1100 (±81)	17,000	~7.0
Zi£268		8.1(±1.8)	8.1(±1.8) 1000(±120)	130,000	8

OPHIUM BUCCOST.

FIG. 10 Selections for the HIV promoter site

TAG DNA target site **TCG** ACG

-1 12356-1 12356-1 12356

						_
	"wild-type"		ASADTR NRSDSR	TSSNKK		
	HP1	LRADDN	LRADDN LSQTKR IRGNVR	IRGNVR		
	HP2	AKADDR	AKADDR LSQTKR VKSNRR	VKSNRR		
	нрз	LRADDR	LRADDR LSQTKR IGSNRR	IGSNRR		
	HP4	LRADDR	LRADDR LSQTKR VKSNRR	VKSNRR		
	нр5	LRTDDR	LRTDDR LSQTQR LNSNAR	LNSNAR		
4	нре	LRTDDR	LRTDDR LSQTRR LRSNGR	LRSNGR		
\	HP7	LRADDR	LRADDR LSQTKR MRSNMR	MRSNMR		
	нрв	LRADDR	LRADDR LRQTKR LRANLR	LRANLR		
	6ан	LRADDR	LRADDR LAQTKR	IGSNTR		
	HP10	LRTDDR	LRTDDR LSQTNR LQGNKR	LQGNKR		
	HP11	LRADDR	LRADDR LRQTKR LRANLR	LRANLR		
4	HP12	NNAMVR	NNAMVR LSQTQR MQGNSR	MQGNSR		_
\					0 1 2 3 4 5 8 7 8	

fold-activation

FIG. 11 In vitro characterization of HIV Promoter ZFPs

Protein	Sequence	K _{apec} (pM)	K _d non-spec Sp (nM)	ecificity ratio	# of Specificity DNA bases ratio specified
"wt"	ASADTR NRSDSR I	SSNKK Unable t	o calculate	(does not	NRSDSR TSSNKK Unable to calculate (does not bind in vitro)
HP6	LRTDDR LSQTRR L	LSQTRR LRSNGR 9.3 (±1.2) 820 (±74)	820 (±74)	87,000	~8.2
HP12	NNAMVR LSQTQR MQGNSR 9.3 (±0.39)180 (±8.8)	IQGNSR 9.3 (±0.3	9)180 (±8.8)	19,000	~7.1
Zi£268		8.1 (±1.8	8.1(±1.8) 1000(±120) 130,000	130,000	8 .5